

PROJECT NUMBER: 1720  
PROJECT TITLE: Physiochemical Morphology  
PERIOD COVERED: November 1-30, 1985  
PROJECT LEADER: E. Thomas  
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Objective: To determine the biochemical and biophysical properties of chloroplast submembrane preparations with respect to oxygen evolution and elucidate the degradation pathways of chloroplast proteins as a function of senescence. (V. Baliga and H. Nakatani)

Status:

Chloroplasts of a clover mutant could not be detergent fractionated in terms of PSII and PSI. This suggests a different structural localization of the photosystems in the membrane the mutant versus that in wild-type material. V. Baliga presented the results of the clover studies at Philip Morris R&D and at the First Intl. Congress of Plant Molecular Biology (Savannah, Georgia, Oct.27-Nov.2,1985). Radiolabelling ( $^{125}\text{I}$  and  $^3\text{H}$ ) studies on PS II submembrane preparations have been approved at MCV (Dr. Larry Shook) through the coordinated efforts of A. Frisch, R. Izac, B. Edwards and B. Davies. MCV facilities will also be used to conduct control experiments on non radioactive preparations. Results of studies on tobacco chloroplast senescence show a decrease in the LHCII complex (SDS-PAGE profile) which was correlated with an increase in the chlorophyll a/b ratio. Decrease of the alpha and beta subunits of the coupling factor were found and correlated with the inability of the chloroplast to form a pH gradient. Corrected absorption spectra for chlorophylls a and b (Sigma) were obtained and the wavelength drives of the Farrand Fluorimeter were recalibrated.

Plans: Dr. Andrew Staehelin (University of Colorado, Boulder) has agreed to collaborate in studies to examine the topological architecture of PS II and PS I by immunocytochemical techniques. Antibodies are to be provided by Dr. N.H. Chua (Rockefeller University, New York) and Dr. C.J. Arntzen (DuPont, Wilmington, Delaware). Trifluoromethyl( $^{125}\text{I}$ )iodophenyldiazurine will be tested initially to label Ca-sensitive proteins in PS II submembrane preparations associated with oxygen evolution and examined by autoradiography at MCV. Further studies are being continued to reduce the LHCII component in the PS II preparations.

The variability of damaged photosynthetic components in the senescence study suggests a requirement to monitor the structural integrity (electron microscopy) of the cellular matrix as a function of senescence rather than to rely simply on leaf coloration and position.

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Evaluation of isolation and assay methods for carboxypeptidase and leucine aminopeptidase in Project 1904 have been made to try to optimize activity measurements.

Objective: Study the physical and chemical properties of green tobacco and relate them to the mechanical properties of cured leaf. (E. Taylor, E. Thomas, J. Lyle, P. Echlin)

Status: Sixty of the 102 leaves from the summer of '85 greenhouse growth study have been analyzed for elemental distribution using the ZAF/PB ratio method. All of the leaves from the Sr and Rb-spiked tobacco plants and control leaf materials have been examined by EDS. Digimapping was also performed on the control leaf material for Ca and K, and on Sr and Rb-spiked material. (Note: Sr and Rb are assumed to be biologically analogous to Ca and K, respectively.) The intensities of K and Rb were closely correlated and were localized in the vacuoles of the upper epidermal cells. The cell wall had relatively little of these latter two elements. Sr and Ca were distributed uniformly throughout the different tissue types. An LVDT (linear variable differential transformer) was obtained from S. Ganeriwala and calibrated to obtain strain measurements with the tensile stage apparatus. The Young's modulus for a rubber sample was determined and agreed with published values. Jackie Lyle attended a course on electron microscopy and X-ray microanalysis at the State University of New York, New Paltz, N.Y. (S. Edith Taylor is providing on-going training on STEM and TEM to J. Lyle at PM).

Plans: The data for the summer leaf material and the Sr and Rb-treated material are to be stored in the computer matrix for further analysis. Continue tensile stage studies on tobacco material with experiments to determine the Young's modulus on Bright, ripe tobacco leaves.

Service Work:

Soot samples from the parking lot area and neighboring stemmery have been examined by EDS for Bob Carpenter: a package of punctured Va Slim cigarettes was visually examined. No discoloration nor residue (by EDS) was found on the paper or tobacco. The hole sizes were determined to be 750 +/- 50 microns.

Polyclonal antibodies raised against nitrate reductase (NR) from squash have been obtained from Dr. W. Campbell (Michigan Technological University) to attempt to identify the presence of NR in various tobacco samples (Project 6902) by Western blotting techniques (this technique assumes that the antibody against the squash NR will react with the tobacco NR).

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